28. (Once Amended) A method according to claim 1 [wherein the assay determines] comprising determining whether the treated test cells have undergone reversion of a neoplastic phenotype, differentiation or apoptosis.

30. (Once Amended) A method according to claim [6] 29 [wherein the assay determines] comprising determining whether the treated test cells have undergone reversion of a neoplastic phenotype, differentiation or apoptosis.

Remarks

Applicants respectfully request reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein.

Applicants have added no new matter by their amendments. Support for the amendment of claim 1 is found within the specification at page 4, line 20-23. Support for the amendment of claims 28 and 30 is found within the specification at page 10, line 20-23 and page 14-15, line 17-6.

Claim Rejections, 35 U.S.C. § 112

The Examiner rejected claims 1-4 under 35 U.S.C. § 112, second paragraph, alleging that the claims are indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Examiner asserts that the method of claim 1 does not provide a correlative recitation to relate increased levels of micronucleation with a decrease in the presence of double minute chromosomes or extrachromosomal DNA.

Applicants respectfully respond that the amendment of claim 1 obviates the Examiner's rejections.

The Examiner rejected claims 28 and 30 under 35 U.S.C. § 112, second paragraph, alleging that the claims are indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Examiner asserts that it is not clear to what biological process "reversion" refers. The Examiner also asserts that claims

28 and 30 should be rewritten in independent form alleging that the claims change the endpoint of the assay step as recited in the independent claims from which they depend. The Examiner further asserts that, for example, claim 28 does not contain all of the limitations of claim 1 from which it depends.

Applicants have amended claims 28 and 30 to address the Examiner's rejection.

Applicants respectfully wish to state that claims in dependant form are construed to include all the limitations of the claim incorporated by reference into the dependent claim. M.P.E.P. § 608.01(n). Therefore, claims 28 and 30 are construed to contain all of the claim limitations of claims 1 and 29.

Applicants submit that the above comments and amendment of claims 28 and 30 obviate the Examiner's rejections.

It is respectfully submitted that the pending claims are in compliance with the requirements of 35 U.S.C. § 112, second paragraph. Therefore, withdrawal of the rejections of the claims under 35 U.S.C. § 112, second paragraph is respectfully requested.

Claim Rejections, 35 U.S.C. § 103(a)

The Examiner rejected claims 1, 3, 28, 29 and 30 under 35 U.S.C. § 103(a) as being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed.

Eckhardt disclosed that hydroxyurea (HU) can eliminate amplified copies of c-myc located on double minute chromosomes (DMs) which can lead to a reduction in tumorigenicity in vitro and in vivo (abstract). HU and dimethyl sulfoxide (DMSO) were reported to reduce both c-myc gene copy number and expression, and induced differentiation in cells containing c-myc amplified on DMs through elimination of the DMs (abstract). However, while both DMSO and HU caused a reduction in the number of DMs, only HU induced the formation of micronuclei (page 6677). Eckhardt further disclosed that retinoic acid (RA) and DMSO eliminate DMs through a mechanism that does not involve micronuclei formation (pages 6674, 6677 and Table 2).

Snapka disclosed a strategy for growing mouse cells that were unstably MTX-resistant in the absence of methotrexate and monitoring the decrease in their DHFR gene dosage as a function of cumulative cell doublings in the presence of a putative "gene loss-inducing" agent (page 7534). Hydroxyurea was shown to increase the rate of loss of the DHFR genes from these cells through use of this strategy. However, the strategy did not indicate if genes were eliminated though formation of micronuclei or through other elimination mechanisms not involving micronuclei formation, such as that caused by DMSO. Snapka also reported indistinguishable results when gene loss was analyzed with either nuclear or whole-cell DNA (page 7536, Figure 5). These results indicate that the mechanism of DHFR gene loss was unknown.

Neither Eckhardt or Snapka, alone or in combination, describe or suggest a method for identifying an agent that causes loss of double minute chromosomes or extracellular DNA from a cell through a mechanism involving micronucleation and then loss of the DNA. Additionally, neither reference alone or in combination describes a method for identifying an agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells.

It is also respectfully submitted that the disclosures of Snapka and Eckhardt would lead one of skill in the art away from the claimed invention because the combination teaches that extrachromosomal DNA is lost through mechanisms that do not involve micronucleation. Eckhardt disclosed that HU, retinoic acid and DMSO function through different mechanisms to reduce c-myc expression and that HU was the only agent that induced micronuclei formation (pages 6676-6677). The search described by Snapka was for treatments that accelerate loss of amplified genes from mammalian cells, but the search did not specifically select for treatments that caused micronuclei formation followed by loss of the micronuclei. Rather, Snapka reported that the mechanism for loss of extrachromosomal DNA was not certain (page 7535-7536). Therefore, because the references teach that extrachromosomal DNA is eliminated from cells through mechanisms that do not involve micronucleation, the combination would lead one skilled in the art away from the claimed invention.

Because the references alone or in combination do not teach the claimed invention and because the combination would teach away from the claimed invention, it is respectfully requested that the Examiner withdraw the rejection under 35 U.S.C. § 103.

The Examiner rejected claims 1, 3, 4, 28, 29 and 30 under 35 U.S.C. § 103(a) as being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci., (USA), 80: 7533-7537 (1983)) and further in view of Dertinger et al. (U.S. Patent 5,858,667; published January 12, 1999; filed September 6, 1996). This rejection is respectfully traversed.

Eckhardt and Snapka are described above.

Dertinger describe a single-laser flow cytometric method for determining whether a compound produces micronucleation in erythrocyte populations (Example 5). Dertinger does not disclose or suggest a method for identifying agents that decrease the presence of either double minute chromosomes or extracellular DNA in a cell through a mechanism involving micronucleation and then loss of the DNA. Also, Dertinger does not disclose or suggest a method for identifying an agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells.

Eckhardt, Snapka and Dertinger, alone or in combination, do not describe or suggest a method for identifying agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Also, Eckhardt, Snapke and Dertinger, alone or in combination, do not describe or suggest a method of identifying a therapeutic agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells. For the aforementioned reasons, the cited references do not make the claims of the invention obvious under 35 U.S.C. § 103(a). Therefore, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

The Examiner rejected claims 1, 2, 28, 29 and 30 under 35 U.S.C. § 103(a) as being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci., (USA), 80: 7533-7537 (1983)) and further in view of Livingstone et al. (Livingstone et al., Cell, 70: 923-935 (1992)).

This rejection is respectfully traversed.

Eckhardt and Snapka were described above.

Livingstone examined whether the mutation or loss of one or both p53 alleles was sufficient to allow the amplification of CAD (trifunctional enzyme carbamoyl-P synthetase, aspartate transcarbamylase, dihydroorotase) to occur (page 923). They reported that cells retaining one wild-type p53 allele mimicked the behavior of primary diploid cells: the cells arrested growth in the presence of drug and failed to demonstrate amplification. Cells losing the second p53 allele failed to arrest when placed in N-(phosphonacetyl)-L-aspartate (PALA) and displayed the ability to amplify at high frequency (abstract). However, Livingstone disclosed that even though two tumorigenic cell types appeared to exhibit a wild-type p53 status, the cells demonstrated measurable potentials to amplify the endogenous CAD gene with frequencies of $2x10^{-5}$ (U2-OS) and $1x10^{-3}$ (RKO) (Table 3). This was interpreted to indicate that loss of p53 is not necessary for gene amplification to occur (page 927). Livingstone also disclosed that the data taken together suggested that p53 is a critical component in a pathway that regulates gene amplification, but also disclosed that alternate pathways, possibly including deficiencies in other steps of the same pathway, can also modulate gene amplification frequency (page 931). Various possibilities were proposed such as, alternate ways of acquiring the ability to amplify genes and that p53 is not the sole regulator of gene amplification. Also proposed were proteins such as viral oncoproteins and cellular proteins that bind to p53 and alter its function, and pathways that bypass p53 circuitry and inactivation of p53 by other mechanisms (page 931).

Because multiple pathways that do not involve p53 are thought to affect gene amplification, one skilled in the art would not be led to use a cell lacking a functional tumor suppressor gene in a method for identifying a therapeutic agent that produces a test cell having an increased level of micronucleation and decreased level of double minute chromosomes or extrachromosomal DNA. Also, Livingstone does not disclose or suggest a method for identifying an agent that decreases the presence of double minute chromosomes or extracellular DNA in a cell through a mechanism involving micronucleation and loss of the DNA. Livingstone also does not disclose or suggest a method for identifying an agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells.

Eckhardt, Snapka and Livingstone, alone or in combination, do not describe or suggest a method for identifying agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Also, Eckhardt, Snapke and Livingstone, alone or in combination, do not describe or suggest a method of identifying a therapeutic agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells. For the aforementioned reasons, the cited references do not make the claims of the invention obvious under 35 U.S.C. § 103(a). Therefore, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

The Examiner rejected claims 1, 3, 4 and 29 under 35 U.S.C. § 103(a) as being unpatentable over Shimizu et al. (Shimizu et al., Nature Genetics, 12: 65-71 (1996)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed.

Snapka was previously described.

Shimizu reported a method for purifying double minute chromosomes (DMs) from micronuclei isolated from a cell (abstract). The method involves the use of HU to induce micronuclei formation and then separation of double minute chromosomes from the micronuclei for use as probes to determine gene dosage and the chromosomal location that generated the double minute chromosomes (page 67 and Figure 2). Shimizu does not disclose or suggest a method for screening agents for their ability to decrease the presence of double minute chromosomes or extrachromosomal DNA in a cell. Also, Shimizu does not disclose or suggest a method for identifying an agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells.

It is respectfully submitted that the disclosure of Shimizu does not lead one of skill in the art to the claimed invention because Shimizu related that an important goal of DM purification is the isolation of DNA of sufficient quality and quantity to prepare FISH probes to enable identification of the chromosomal location(s) that generated the DMs (page 67). The claimed invention is directed toward methods to identify agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double

minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Thus, the disclosure of Shimizu leads one of skill in the art away from the claimed invention.

Shimizu and Snapka, alone or in combination, do not describe or suggest a method for identifying agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Also, Shimizu and Snapka, alone or in combination, do not describe or suggest a method of identifying a therapeutic agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells. For the aforementioned reasons, the cited references do not make the claims of the invention obvious under 35 U.S.C. § 103(a). Therefore, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Double Patenting Rejection

The Examiner rejected claims 1, 3, 4 and 29 under the judicially created doctrine of obviousness-type double patenting over claims 1-6 of Wahl et al. (U.S. Patent 6,033,849; filed August 26, 1996) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed.

Snapka was described previously.

Wahl reported a method for isolating and identifying an extrachromosomal amplified target nucleic acid from micronuclei in a cell. The method involves contacting a cell suspected of having extrachromosomal amplified target nucleic acid with a non-alkaloid agent capable of inducing the formation of micronuclei in the cell, isolating the micronuclei from the cell, isolating the extrachromosomal target nucleic acid from the isolated micronuclei and hybridizing the nucleic acid from the isolated micronuclei with chromosomal DNA from the cell, wherein the isolated micronuclei nucleic acid is a probe, and identifying the amplified target nucleic acid (Figure 1). Wahl does not disclose or suggest a method for screening agents for their ability to decrease the presence of double minute chromosomes or extrachromosomal DNA in a cell. Also, Wahl does not disclose or suggest a method for identifying an agent for treatment of neoplastic

cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells.

It is respectfully submitted that the disclosure of Wahl does not lead one of skill in the art to the claimed invention because Wahl related a method to isolate extrachromosomally amplified genes from a cell while the invention relates to a method to identify agents that cause elimination of double minute chromosomes and extrachromosomal DNA from a cell. Thus, the disclosure of Wahl leads one of skill in the art away from the claimed invention.

Wahl and Snapka, alone or in combination, do not describe or suggest a method for identifying agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Also, Wahl and Snapka, alone or in combination, do not describe or suggest a method of identifying a therapeutic agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells. Accordingly, it is respectfully requested that the Examiner withdraw the obviousness-type double patenting rejection.

Claims 1, 3, 4 and 29 were rejected under 35 U.S.C. § 103(a) as being obvious over Wahl et al. (U.S. Patent 6,033,849; filed August 26, 1996). This rejection is respectfully traversed.

Applicant notes that the citation for the Snapka disclosure used below is not listed in the instant rejection. Therefore, Applicant used the previously indicated Snapka et al. reference (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)) in responding to the Examiner.

Wahl and Snapka were described previously.

It is respectfully submitted that the disclosure of Wahl does not lead one of skill in the art to the claimed invention because Wahl related a method to isolate extrachromosomally amplified genes from a cell while the invention relates to a method to identify agents that cause elimination of double minute chromosomes and extrachromosomal DNA from a cell. Thus, the disclosure of Wahl leads one of skill in the art away from the claimed invention.

Furthermore, Wahl and Snapka, alone or in combination, do not describe or suggest a method for identifying agents that cause a decrease of double minute chromosomes or extrachromosomal DNA within a cell through micronucleation of the double minute chromosomes or extrachromosomal DNA and elimination of the micronuclei from the cell. Also, Wahl and Snapka, alone or in combination, do not describe or suggest a method of identifying a therapeutic agent for treatment of neoplastic cells that causes an increased level of micronucleation in treated cells relative to that of untreated cells. Therefore, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-371-2123) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

GEOFFREY M. WAHL ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 371-2123

Date 7/30/01 By _____
Leif Stordal
Reg. No. 46,251

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 30th day of July, 2001.

Dawn M Poole
Name

Dawn M. Poole
Signature